



## Variant Creutzfeldt-Jakob disease

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It is clear that the prion strain causing bovine spongiform encephalopathy (BSE) in cattle has infected human beings, manifesting itself as a novel human prion disease, variant Creutzfeldt-Jakob disease (vCJD). Studies of the incubation periods seen in previous epidemics of human prion disease and of the effect of transmission barriers limiting spread of these diseases between species, suggest that the early variant CJD cases may have been exposed during the preclinical phase of the BSE epidemic. It must therefore be considered that many cases may follow from later exposure in an epidemic that would be expected to evolve over decades. Since the number of people currently incubating this disease is unknown, there are concerns that prions might be transmitted iatrogenically via blood transfusion, tissue donation, and, since prions resist routine sterilisation, contamination of surgical instruments. Such risks remain unquantified. Although variant CJD can be diagnosed during life by tonsil biopsy, a prion-specific blood test is needed to assess and manage this potential threat to public health. The theoretical possibility that BSE prions might have transferred to other species and continue to present a risk to human health cannot be excluded at present.

The transmissible spongiform encephalopathies, or prion diseases, a group of neurodegenerative diseases that affect human beings and animals, have attracted much public and media attention. The unique biology of these previously obscure brain diseases have been the subject of long-standing and intense research and controversy. However, the appearance and rapid evolution to epidemic of the novel animal prion disease bovine spongiform encephalopathy (BSE), with the legitimate concerns of human transmission and a potentially severe threat to public health, have placed these diseases, and the people who have managed and studied them, under an unprecedented political and inquisitorial spotlight.

The human prion diseases have been traditionally classified into Creutzfeldt-Jakob disease (CJD), Gerstmann-Strausler-Scheinker disease, and kuru, and can be divided into three aetiological categories: sporadic, acquired, and inherited. The acquired prion diseases include iatrogenic CJD and kuru, and arise from accidental exposure to human prions through medical or surgical procedures or participation in cannibalism. Epidemiological studies show no association between sheep scrapie and the occurrence of CJD in human beings.<sup>1</sup> Sporadic CJD occurs in all countries, with a random case distribution and an annual incidence of one per million. Around 15% of human prion disease is inherited, all cases to date have been associated with coding mutations in the prion protein gene (*PRNP*), of which more than 20 distinct types are recognised.<sup>2</sup> The inherited prion diseases can be diagnosed by *PRNP* analysis, and the use of these definitive genetic diagnostic markers has enabled recognition of a wider phenotypic spectrum of human prion disease, which includes a range of atypical dementias and fatal familial insomnia.<sup>3-5</sup> No such pathogenic *PRNP* mutations are present in sporadic and acquired prion disease. However, a common prion

protein (PrP) polymorphism at residue 129 (where methionine or valine can be encoded) is a key determinant of genetic susceptibility to acquired and sporadic prion diseases, which occur mostly in homozygous individuals.<sup>6-8</sup> This protective effect of *PRNP* codon 129 heterozygosity is seen also in some of the inherited prion diseases.<sup>9,10</sup>

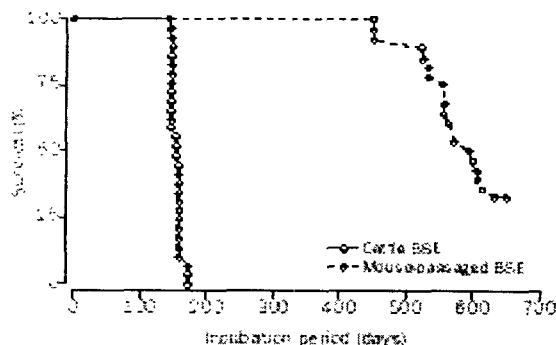
Prion diseases of human beings and animals are associated with the accumulation in the brain of an abnormal partially protease-resistant isoform of a host-encoded glycoprotein known as prion protein. The disease-related isoform, PrP<sup>Sc</sup>, is derived from its normal cellular precursor, PrP<sup>C</sup>, by a post-translational process that involves a conformational change. PrP<sup>C</sup> is rich in  $\alpha$ -helical structure, whereas PrP<sup>Sc</sup> seems to be composed mainly of a  $\beta$ -sheet structure. According to the "protein-only" hypothesis,<sup>11</sup> an abnormal PrP isoform<sup>12</sup> is the principal, and possibly the sole, constituent of the transmissible agent or prion. PrP<sup>Sc</sup> is postulated to act as a conformational template that promotes the conversion of PrP<sup>C</sup> to further PrP<sup>Sc</sup>. PrP<sup>C</sup> seems to be poised between two radically different folding states, and  $\alpha$  and  $\beta$  forms of PrP can be interconverted in suitable conditions.<sup>13</sup> Soluble  $\beta$ -PrP aggregates in physiological salt concentrations to form fibrils with morphological and biochemical characteristics similar to PrP<sup>Sc</sup>. A molecular mechanism for prion propagation can be proposed.<sup>13</sup> Prion replication, with recruitment of PrP<sup>C</sup> into the aggregated PrP<sup>Sc</sup> isoform, may be initiated by a pathogenic mutation (resulting in a PrP<sup>C</sup> predisposed to form  $\beta$ -PrP) in inherited prion diseases, by exposure to a "seed" of PrP<sup>Sc</sup> in acquired disease, or as a result of the spontaneous conversion of PrP<sup>C</sup> to  $\beta$ -PrP (and subsequent formation of aggregated material) as a rare stochastic event in sporadic prion disease.

Although the existence of multiple strains or isolates of prions with distinct biological properties has provided a challenge to such a protein-only model of prion replication, prion strains can be clearly distinguished by differences in the biochemical properties of PrP<sup>Sc</sup>.<sup>14-18</sup> Prion-strain diversity seems to be encoded by differences in PrP conformation and pattern of glycosylation.<sup>16</sup> A molecular approach to strain typing based on these characteristics has enabled the identification of four main types of CJD—sporadic and iatrogenic CJD are of PrP<sup>Sc</sup>

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#### Typical effect of a species barrier: transmission of cattle BSE and mouse-passaged BSE to inbred FVB mice

Primary passage associated with long mean incubation period, wider range of incubation periods, and <100% animals developing clinical disease. Subsequent passages in mice associated with consistent short incubation and all animals succumb to disease

and an appreciation of the biological effects of species barriers, does, in my opinion, challenge such optimism.

#### Epidemics of human prion disease

The kuru epidemic in the eastern highlands of Papua New Guinea provided the largest experience of acquired human prion disease.<sup>34</sup> Kuru was transmitted during cannibalistic feasts when deceased relatives were eaten by their close family and others in the immediate community. The epidemic is thought to have originated when a person who had developed sporadic CJD, which is known to occur at random in all populations, died and was eaten. The recycling of prions within this isolated population led to a substantial epidemic that became the major cause of death among children and adult women. Before the end of cannibalism in the late 1950s, such feasts were common and the multiple exposures that individual kuru patients may have had complicated precise estimates of incubation periods. However, studies of later cases with well-defined exposures provided more precise estimates.<sup>35</sup> Rare cases of kuru were recorded in children as young as 4-5 years, which suggests incubation periods of this length or shorter. Although dietary exposure to kuru was assumed to be the main route of transmission, inoculation with brain or other tissue, either via cuts or sores or to the conjunctiva (through eye rubbing) was also likely. Since such routes of transmission in laboratory animals result in shorter mean incubation periods than oral exposure, these cases of kuru with very short incubation periods may not represent oral transmission. At the other extreme, occasional cases of kuru are still occurring in the Fore region in patients exposed during some of the last cannibalistic feasts held in their villages, and are consistent with incubation of longer than 40 years (unpublished data). Mean incubation periods have been estimated to be around 12 years (M Alpers, personal communication).

More than 100 cases of acquired CJD have occurred as a result of intramuscular injection with human cadaveric pituitary-derived growth hormone, inadvertently contaminated with CJD prions. Multiple exposures, commonly over several years, complicate accurate estimation of incubation periods. Mean incubation periods are, however, estimated to be about 12 years.<sup>36</sup>

Together, these data suggest that incubation periods of human prions in human beings (in the absence of a

species barrier) after peripheral inoculation or oral exposure, range from at least 4 years to 40 years, with a mean of about 10-15 years. Cases resulting from oral exposure may have longer mean incubation. Most of the earliest cases of CJD related to iatrogenic growth hormone in the UK were homozygous for valine at polymorphic *PRNP* codon 129, a genotype seen in about 11% of normal white people.<sup>37</sup> This effect of codon 129 genotype may affect the mean incubation period as well as overall susceptibility. Heterozygotes would be expected to have the longest mean incubation periods.

#### The species barrier

Transmission of prion diseases between different mammalian species is limited by a so-called species barrier.<sup>38</sup> On primary passage of prions from species A to species B, typically not all inoculated animals of species B would succumb; those that did would do so with longer and more variable incubation periods than with transmission of prions within the same species, on which, typically, all inoculated animals would succumb with a short and remarkably consistent incubation period. On second passage of infectivity to further animals of species B, transmission parameters resemble within-species transmissions, with most, if not all, animals developing the disease with short and consistent incubation periods. Species barriers can therefore be quantified by measurement of the fall in mean incubation period on primary and second passage in the new host species or, perhaps more rigorously, by comparative titration study. The latter involves inoculation of serial dilutions of an inoculum in the donor and the new host species, and comparison of the dilution required to kill 50% of animals ( $LD_{50}$ ). The effect of a substantial species barrier (for instance that between hamsters and mice) is that few, if any, animals succumb to disease on primary passage and at incubation periods approaching the natural lifespan of the species concerned.

As an example, consider the species barrier that limits transmission of BSE from cattle to conventional mice. This barrier has been extensively studied experimentally because of the use of mice to assay BSE infectivity. BSE can be readily transmitted to mice, with most, if not all, inoculated animals succumbing to disease on primary passage (also known as a high attack rate). This relatively moderate species barrier has been formally measured by comparative titration studies of the same BSE isolate by intracerebral inoculation into cattle and mice, which have shown a barrier of about 1000-fold (ie, it takes 1000 times more BSE inoculum to kill a mouse than a cow).<sup>39</sup> The effect of this barrier on incubation periods is to increase mean incubation periods by around three-fold and to substantially increase the range of incubation periods (figure).

Such experiments are generally done by the most efficient route of transmission (intracerebral). A formal titration of BSE-infected material in mice to determine an oral  $LD_{50}$  has not been reported. However, oral challenge with about 10 g of BSE-affected cow brain killed most exposed mice.<sup>40</sup> If the bovine-to-human species barrier were similar to that to mice, that would suggest an oral  $LD_{50}$  in human beings of a similar magnitude to that for mice. Clearly, the hope is that the species barrier limiting transmission of BSE to human beings will be far higher. If we assume, however, that the barrier is similar (it remains possible that it could be lower), extrapolation would

bovine offals ban was being introduced and the incidence of BSE in cattle was still rising rapidly. The exposure of the population to BSE depended, however, not only on the BSE epidemic curve itself and the timing of the specified bovine offals and other statutory bans designed to keep exposure to BSE to a minimum, but also on the extent to which these bans were effective. The effect of such bans is dependent on the extent to which high-titre tissues, especially brain, were actually used for human food products before the ban, much of this material may have been rendered for animal feed. Although it could be argued that the current cases of variant CJD were exposed to BSE near the peak period of exposure, perhaps around 1990, incubation periods of the earliest variant CJD cases would have to be 5 years or less, which corresponds to the shortest reported incubation periods of kuru, for which transmission did not involve a species barrier. This possibility seems implausible and contradictory, since cases arising from earlier exposure to BSE would then be expected to have occurred. Shorter incubation periods imply a correspondingly smaller species barrier, which in turn implies a larger epidemic size for a given population exposure. Arguments for longer incubation periods in the current variant CJD patients, with apparently reassuring implications for the species barrier and attack rate, have to place infection at a very early stage of the BSE epidemic when exposure was low compared with subsequent exposure.

Another important consideration is that the affected individuals to date have had no history of an unusual dietary or occupational exposure to BSE.<sup>28</sup> Transmission of prion diseases is highly dose dependent, and inoculation is a more efficient route of transmission than is the oral route in laboratory animals. Therefore, if BSE had low pathogenicity for human beings and only a small epidemic occurred, the few individuals who contracted variant CJD might be expected to have had inoculation injuries with BSE-infected material (eg, abattoir workers) or an unusual diet with known exposure to brain or other high-titre tissues. Instead, the lack of such history is suggestive of one or more key environmental cofactors or something unusual about these individuals that gives them a high innate sensitivity to BSE. Such susceptibility could be an increased sensitivity to infection with the agent, a shorter incubation period after infection, or both. The unremarkable history of exposure to BSE among patients with variant CJD to date suggests that these susceptibility factors are more important than the degree of exposure. Susceptibility could be genetic or related to one or more cofactors. All patients with variant CJD analysed to date have been *PRNP* codon 129 methionine homozygotes (refs 45, 46, and unpublished data). All cattle studied are homozygous for methionine at the corresponding bovine codon.<sup>47</sup> About 38% of the normal white population are, however, of this *PRNP* genotype.

Studies of inbred lines of mice, encoding the same *Prnp* allele, but with various incubation periods to the same prion strain, suggest that other genetic loci affect the incubation period. The human homologues of these unidentified disease-modifier loci are likely to be relevant to BSE incubation periods in human beings. Possible cofactors that might facilitate infection by the oral route include buccal lesions and tonsil and gastrointestinal infection. Coexistent gut infection with nematodes may predispose to scrapie infection of sheep.<sup>48</sup> It has been long

established in natural sheep scrapie and experimental rodent scrapie that early prion replication occurs in the lymphoreticular system, with detectable neuroinvasion much later in the incubation period.<sup>49</sup> In cattle, BSE infectivity is first detectable in the distal ileum around 6 months after exposure, consistent with infection in Peyer's patches.<sup>50</sup> PrP<sup>Sc</sup> is detectable in lymphoreticular tissues from all patients studied to date with variant CJD, in sharp contrast to other forms of CJD, which suggests a prominent lymphoreticular phase in human infection with BSE prions.<sup>46,51,52</sup> Such a cofactor could be relevant to the unexplained age distribution of variant CJD, since children would be expected to have more frequent infections activating such lymphoreticular tissues that may facilitate access, replication, or both of BSE prions. By contrast, immunosuppression may be protective against prion disease.<sup>53,54</sup>

To date, epidemiological studies have not identified any environmental risk factors for variant CJD other than that of UK residence. Identification of particular foodstuffs that might have contained high concentrations of the BSE prion and have caused variant CJD is severely hampered by lack of knowledge about the distribution of BSE-infected tissues in food products and the extreme difficulty of obtaining accurate and detailed dietary histories from years previously in anyone, let alone those with severe cognitive impairment. However, some hypothesis-driven questions could be pursued, for example, about histories of specific infectious diseases (that result in activation of the lymphoreticular system of the tonsils, gut, or both—or severe leucocytosis) and gastrointestinal disorders (that might affect gut permeability to prions) during the peak exposure of the population to BSE. These questions will also be challenging to an epidemiological approach, and experimental studies to confirm such cofactors in laboratory animals may be the only realistic way to address such hypotheses.

Finally, transmission studies in transgenic mice expressing human PrP valine 129 have suggested that transmission of BSE to human beings with the *PRNP* codon 129 valine homozygous genotype (and possibly heterozygotes) might lead to a clinical syndrome distinct from variant CJD, since a different PrP<sup>Sc</sup> type (designated type 5) was produced on transmission of variant CJD to such animals.<sup>30</sup> It is unknown whether type 5 human prions would produce another new disease, such as variant CJD, which was quite distinct and easy to differentiate from classic CJD on clinical and pathological criteria, or produce a phenotype indistinguishable from classic forms of CJD. In the latter situation, such cases could still be distinguished by molecular-strain typing. No such cases have yet been reported.

### Remaining routes of transmission of BSE and variant CJD

The substantial extension of measures to limit dietary exposure to BSE prions in March, 1996, especially the 30-month rule (whereby only animals younger than this age can be used for human foodstuffs), allied with the continued decline in the UK BSE epidemic, should have ensured that any cattle BSE entering the human diet is kept to a minimum, if significant at all, compared with earlier exposure. It is still theoretically possible, however, that BSE could have been transmitted to other

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